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The Effects of Dietary Saponins on Ruminal Methane Production and Fermentation Parameters in Sheep: A Meta Analysis

Review Article

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ABSTRACT

Ruminants production systems are facing a critical period within global agriculture due to their unique digestive system which, whilst allowing them to utilize low-quality fiber-rich feed, produces the potent greenhouse gas methane (CH_4) as a by-product. It has been proposed that saponin-rich plants can be used to reduce CH_4 emissions from ruminant livestock, although the reported results are variable in terms of efficacy. Here we use meta-analytical methods to investigate the literature to determine if saponins can contribute to reducing CH_4 production and its further effects on other rumen fermentation parameters in sheep. Following defined search terms available papers on the subject were collected for the period 1990 to 2019 and inclusion and exclusion criteria were applied, an analysis was conducted on CH_4 production, CH_4 per dry matter intake (DMI), ruminal pH, total volatile fatty acid (VFA), acetate, propionate, butyrate, and acetate-to-propionate ratio based on a comparison between a saponin supplemented group and a control group. The standardized effect size (Hedges' *g*) was calculated at the confidence interval of 95%. *Q*-test and I^2 statistic were used to determine heterogeneity and publication bias was identified through the Egger test. The meta-analysis determined that using saponin sources tended to decrease CH_4 production (P=0.062) and acetate-to-propionate ratio (P=0.057), with a reduction in CH_4/DMI (P=0.001) and an increase in propionate concentration (P=0.011). No significant difference was observed in ruminal pH, total VFA concentration, and butyrate concentration. The I^2statistic for the parameters analyzed here was below 50% for heterogeneity with the Egger test results indicating a publication bias for CH_4 production.

KEY WORDS saponin, methane, sheep, meta-analysis.

INTRODUCTION

Methane (CH_4) is a potent greenhouse gas. An often-reported global source is a by-product of enteric fermentation from ruminants, as they utilize low-quality fiber-rich feeds to produce meat, milk, fiber (wool), and a range of valuable co-products (e.g. leather and pharmaceuticals). Methane also represents a significant loss in digestible energy with up to 10% of a feed's gross energy lost during its production (Johnson and Johnson, 1995). Numerous strategies have been proposed to lower CH_4 production in ruminants to reduce the environmental impact of production and enhance animal performance efficiency (Martin *et al.* 2010). There have been many chemical feed additives proposed to lower fermentation-induced CH_4 production in ruminants. However, they often either have toxic effects on the host, reduce significantly fiber digestibility in the rumen or only temporarily act to reduce CH_4, with temporal increases to previous levels observed as the rumen microbiota adapts (Liu *et al.* 2019). Other approaches proposed include the application of plant secondary metabolites such as saponins (Patra *et al.* 2017). Saponins are high molecular weight glycosides in which a triterpene or steroidal aglycone moiety is linked to one or more sugar chains, which can be found in a wide range of plants including *Quillaja saponaria*, *Yucca schidigera*, *Sapindus rarak*, and *Sapindus saponaria* (Wina *et al.* 2005). The number of sugars, the type of sugars, and the stereochemistry of aglycone may vary producing a diverse array of saponins. It has also been suggested that these compounds can mitigate CH_4emission by acting as rumen modifies by lowering the number of protozoa and methanogenic archaea (Patra and Saxena, 2010). Experiments conducted *in vivo* and *in vitro* to examine effects of saponins on CH_4 emissions in ruminants have varied in their response, possibly because of the diverse array of metabolites observed from different plant sources. *In vitro* studies outnumber *in vivo* studies with most conducted on sheep with a smaller number on other ruminants such as goats and cattle. A meta-analysis of effects of saponin-rich sources on CH_4 emissions and rumen fermentation parameters based *in vitro* studies indicated the potential to lower ruminal CH_4 emissions at the same time as altering ruminal VFA patterns with a reduction in acetate and an increase in propionate proportions (Jayanegara *et al.* 2014).

These authors also reported a reduction in the number of protozoa at higher levels of saponin intake (Jayanegara *et al.* 2014). *In vivo* experiments on sheep have revealed that supplementation with *Sapindus saponaria* fruits (Hess *et al.* 2004) or *Yucca schidigera* (Wang *et al.* 2009) reduced CH_4 emissions in sheep. Contrary to these findings supplementation with saponin extract from alfalfa (*Medicago sativa*) root (Klita *et al.* 1996), *Yucca schidigera* (Śliwiński *et al.* 2002), *Quillaja saponaria* (Pen *et al.* 2007), and tea (Camelliaceae; Liu *et al.* 2019) were found to have no significant effect in lowering CH_4 emission compared to a control group.

Furthermore, saponin extracted from *Yucca schidigera* and *Quillaja saponaria* (Pen *et al.* 2007), tea saponin (Yuan *et al.* 2007), and *Yucca schidigera* powder (Santoso *et al.* 2004) have been found to induce no significant reduction in CH_4 per unit of dry matter intake (DMI) when compared to control group while Mao *et al.* (2010) reported a significant decrease in CH_4/DMI for a group receiving tea saponin compared to the control group.

Given these contrasting findings *in vivo*, and the results of the *in vitro* studies' meta-analysis indicating the potential of saponins to lower CH_4 emissions, a similar meta-analysis is required to fully investigate the *in vivo* findings.

Since a meta-analysis can combine and statistically review results from different studies, and further investigate reasons of heterogeneity.

MATERIALS AND METHODS

Literature search and data collection

Literature searches were conducted through databases of ISI Web of Knowledge (http://wokinfo.com) and Google Scholar (http://scholar.google.com) for a period covering January 1990 through to March 2019. The keywords used to search relevant studies included: methane, saponin, and sheep. Several thousand hits were collected from Google Scholar and then the results were saved in order of relevance. After identifying the last relevant record, at least 100 records were saved and then the screening of papers stopped. To identify and collect further relevant papers, the references of the selected papers were evaluated using inter-library links or author correspondence with the aim of finding papers not available in the searched databases.

Inclusion and exclusion criteria

The process resulted in the identification of 149 articles in total. During the first step, we removed duplicate articles (n=48) and review papers (n=30) then identified and included only studies which specifically examined the effects of saponins on methane production, resulting in 49 papers. We then further included papers that contained experiments on sheep with a control group and a group that received saponin. We included studies that measured methane production *in vivo* while removing studies that measured this *in vitro* (n=33) or estimated methane production using equations (n=1). We excluded studies conducted *in vivo* to examine the effects of saponin on methane emission and production parameters in other animals (n=6). A list of the experiments included in the meta-analysis is depicted in Table 1.

Data extraction

The data extracted here included average CH_4 production (g/day), CH_4/DMI, ruminal pH, total VFA, acetate, propionate, butyrate, and acetate-to-propionate ratio. For papers that did not report CH_4/DMI, the value was obtained by dividing CH_4 by DMI. In cases where rumen fermentation parameters were reported temporally, the last reporting time was considered for calculations. We also extracted the standard error of the mean and the number of animals in saponin and control groups. Other data extracted included: author(s) name, year of publication, a method for measuring CH_4 production, breed, and type and dose of saponin used.

No data was extracted for protozoa count since most studies did not report this while others reported the total number of protozoa or number of ciliates only.

Statistical analysis

Statistical analysis was performed using STATA (Intercooled Stata v.13, StataCorp, College Station, TX) and effect size for CH_4 production, CH_4/DMI, and rumen fermentation parameters were calculated in the form of Hedges' *g* at a 95% confidence interval. The effect size is based on the mean difference between treatment and control groups divided by pooled standard deviation and adjusted for bias with small sample sizes (Borenstein *et al.* 2011). Effect sizes are ranked as small, medium and large at 0.2, 0.5, and 0.8 (Cohen, 1988). In this meta-analysis, we also calculated the effect size of the mean difference for CH_4 production and CH_4/DMI. A random-effects model was used (Borenstein *et al.* 2011) where actual effects may vary from one experiment to another, which covers an experiment variable (actual heterogeneity) as well as sampling error.

Forest plots, as a common plot in meta-analyses, were used to present CH_4 production and CH_4/DMI. The forest plot was represented with Hedges'*g* at a 95% confidence interval using a random model.

Heterogeneity

Statistical heterogeneity explains that different effects are found in different studies (Sutton and Higgins, 2008). Chi-square (*Q*) test and the I^2 statistic were determined to measure heterogeneity (Borenstein *et al.* 2011). Variations among the study level were assessed using a *Q* test. Although *Q* test helps identify heterogeneity, the measure I^2 was used to measure heterogeneity (formula 1; Lean *et al.* 2009).

Formula 1: I^2 (%)= Q - (k-1) / Q

Where:
Q: χ^2 heterogeneity statistic.
k: number of trials.
I^2: statistic describes the percentage of variation across studies due to heterogeneity.

The I^2 values of 25%, 50%, and 75% were interpreted as low, medium, and high, respectively. Parameters in which I^2 are greater than 50%, were set as the parameters to indicate significant heterogeneity (Lean *et al.* 2009).

Publication bias

To examine where a publication bias exists we used Egger's linear regression asymmetry. A significant value ($P<0.10$) indicated the presence of bias (Sales, 2011).

RESULTS AND DISCUSSION

Database

Table 1 lists the studies collected and the data used in our meta-analysis. In general, 12 and 15 comparisons were made between control and saponin supplemented groups for CH_4 production and CH_4/DMI, respectively. The number of comparisons for rumen fermentation parameters including ruminal pH, the concentration of total VFA, acetate, propionate, butyrate, and acetate-to-propionate ratio was 15. The studies of Hess *et al.* (2004) and Mao *et al.* (2010) used lambs, Liu *et al.* (2019) conducted experiments on ewes whereas all remaining studies used wethers in their trials. The source of saponin varied across studies with *Sapindus saponaria* fruits in Hess *et al.* (2004), alfalfa root in Klita *et al.* (1996), tea in Yuan *et al.* (2007), Mao *et al.* (2010), and Liu *et al.* (2019), *Yucca schidigera*in Pen *et al.* (2007), Śliwiński *et al.* (2002), Wang *et al.* (2009), and Santoso *et al.* (2004) and finally *Quillaja saponaria*was also used by Pen *et al.* (2007).

CH_4 production and CH_4/DMI

The effect size calculated based on a random model for CH_4 and CH_4/DMI is shown in Table 2, showing a decrease for CH_4 (P=0.062; Figure 1) and CH_4/DMI (P=0.001; Figure 2). Effect size reported as mean difference indicates that using saponin-rich sources reduced CH_4 production by 1.246 g/day and CH_4/DMI by 0.849 g/kg. No heterogeneity was observed for CH_4 production (P=0.142) nor CH_4/DMI (P=0.155) with the Egger test indicating the presence of publication bias for CH_4.

Rumen fermentation parameters

The effect size calculated from the random model together with heterogeneity for rumen fermentation parameters is reported in Table 3. No significant difference was observed between the effect sizes for pH, acetate, and butyrate concentration; while the effect size decreased for propionate concentration and tended to decrease for acetate-to-propionate ratio.

A low heterogeneity was observed for pH, acetate, propionate, and acetate-to-propionate ratio while the heterogeneity for VFA and butyrate was at a medium level. The Egger test results showed no publication bias for rumen fermentation parameters.

A meta-analysis involves the application of defined statistical methods to summarize multiple study results by combining results from these different studies and statistically summarizing the combined results. The current meta-analysis indicated the effectiveness of saponin sources in mitigating CH_4 production (as a trend) and CH_4/DMI in sheep.

Table 1 Summary of papers used for meta-analysis

Reference	NC[1]	Animal	Breed	Saponin products	Response variables
Hess *et al.* (2004)	3	Lamb	Swiss White Hill	*S. saponaria* fruit	CH_4, CH_4/DMI, pH, total VFA, acetate, propionate, butyrate, acetate/propionate
Klita *et al.* (1996)	3	Wether	Suffolk	Alfalfa root	CH_4, CH_4/DMI, pH, total VFA, acetate, propionate, butyrate, acetate/propionate
Liu *et al.* (2019)	1	Ewe	Dorper × thin-tailed Han	Tea saponin	CH_4, CH_4/DMI, pH, total VFA, acetate, propionate, butyrate, acetate/propionate
Mao *et al.* (2010)	1	Lamb	Huzhou	Tea saponin	CH_4/DMI, pH, Total VFA, acetate, propionate, butyrate, acetate/propionate
Pen *et al.* (2007)	2	Wether	Cheviot	*Q. saponaria*; *Y. schidigera*	CH_4, CH_4/DMI, pH, total VFA, acetate, propionate, butyrate, acetate/propionate
Santoso *et al.* (2004)	1	Wether	Cheviot	*Y. schidigera*	CH_4/DMI, pH, total VFA, acetate, propionate, butyrate, acetate/propionate
Śliwińskiet *al.* (2002)	2	Wether	Swiss White Hill	*Y. schidigera*	CH_4, CH_4/DMI, pH, total VFA, acetate, propionate, butyrate, acetate/propionate
Wang *et al.* (2009)	1	Wether	Mongolia	*Y. schidigera*	CH_4, CH_4/DMI, pH, total VFA, acetate, propionate, butyrate, acetate/propionate
Yuan *et al.* (2007)	1	-	Huzhou	Tea saponin	CH_4/DMI, pH, total VFA, acetate, propionate, butyrate, acetate/propionate

[1] NC: No. of comparisons.

Table 2 Effect size and heterogeneity for the effect of saponin on CH_4 production and CH_4/DMI

Outcome	NC[1]	Hedges[2], *g*	95% confidence intervals	P-value	*Q*[3]	P-value	*I*-squared[4], %	MD[5]	95% confidence intervals	Egger
CH_4	12	-0.441	-0.904, 0.022	0.062	15.98	0.142	31.2	-1.246	-2.199, -0.293	0.018
CH_4/DMI	15	-0.671	-1.078, -0.265	0.001	19.26	0.155	27.3	-0.849	-1.304, -0.393	0.344

[1] No. of comparisons.
[2] Standardized unitless effect size for differences between treatment and control groups.
[3] Cochran's Q-values to identify the presence of heterogeneity among studies.
[4] Degree of heterogeneity among studies.
[5] Mean difference.

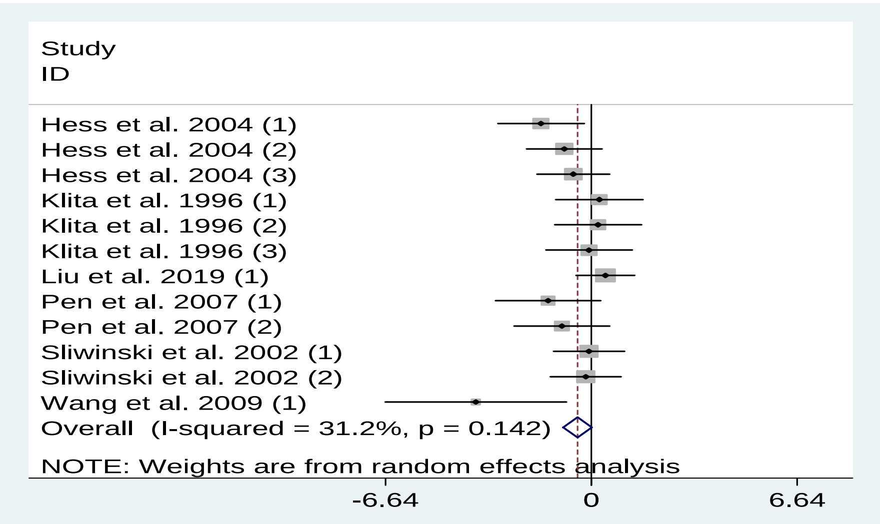

Figure 1 Forest plot of the effect size and 95% confidence interval of the effect of saponin on CH_4 production in sheep. The solid vertical line represents a mean difference of 0 or no effect. Points to the left of the line represent a decrease in CH_4 production and points to the right of the line indicate an increase. Each square around the point effect represents the mean effect size for that study and reflects the relative weighting of the study to the overall effect size estimate. The larger the box, the greater the study contributes to the overall estimate. The upper and lower limit of the line connected to the square represents the upper and lower 95% CI for the effect size

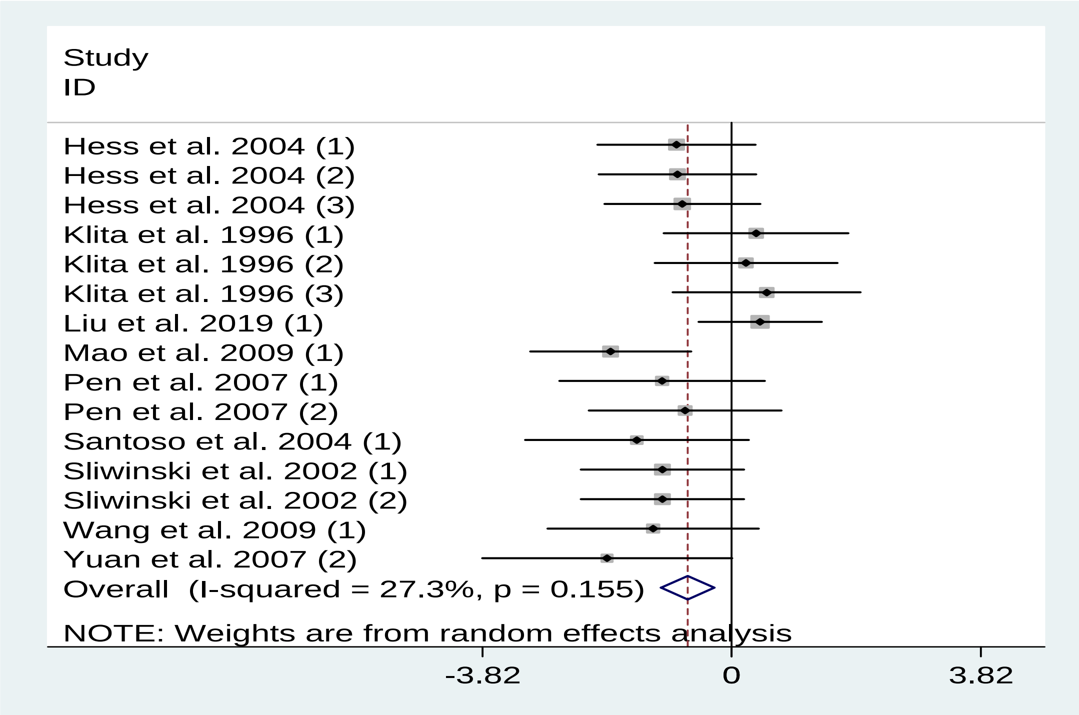

Figure 2 Forest plot of the effect size and 95% confidence interval of the effect of saponin on CH_4/DMI in sheep. The solid vertical line represents a mean difference of 0 or no effect. Points to the left of the line represent a decrease in CH_4/DMI and point to the right of the line indicate an increase. Each square around the point effect represents the mean effect size for that study and reflects the relative weighting of the study to the overall effect size estimate. The larger the box, the greater the study contributes to the overall estimate. The upper and lower limit of the line connected to the square represents the upper and lower 95% CI for the effect size

Table 3 Effect size and heterogeneity for the effect of saponin on rumen fermentation

Outcome	NC[1]	Hedges[2], *g*	95% confidence intervals	P-value	*Q*[3]	P-value	*I*-squared[4], %	Egger
pH	15	-0.135	-0.456, 0.187	0.412	11.38	0.656	0	0.972
Total VFA	15	0.389	-0.086, 0.865	0.108	26.59	0.022	47.4	0.513
Acetate	15	-0.095	-0.416, 0.227	0.564	11.36	0.657	0	0.966
Propionate	15	0.430	0.098, 0.762	0.011	14.20	0.435	1.4	0.238
Butyrate	15	0.326	-0.134, 0.787	0.165	25.34	0.037	44.7	0.165
Acetate/propionate	15	-0.333	-0.674, 0.009	0.057	15.15	0.368	7.6	0.393

[1] No. of comparisons.
[2] Standardized unitless effect size for differences between treatment and control groups.
[3] Cochran's Q-values to identify the presence of heterogeneity among studies.
[4] Degree of heterogeneity among studies.

According to a classification of effect size proposed by Cohen (1988), a medium value for CH_4 production and CH_4/DMI was observed.

These findings, agree with a previous meta-analysis of *in vitro* studies (Jayanegara *et al.* 2014) where saponin-rich sources reduced CH_4 per unit substrate and per total gas produced. However, although the meta-analysis reports a trend for a reduction in CH_4 production several studies reported that using such saponin sources as *Quillaja saponaria* (Pen *et al.* 2007), and *Yucca schidigera* (Śliwiński *et al.* 2002; Pen *et al.* 2007) did not result in a significant reduction, although numerically reductions were observed, which contributed to the meta-analysis result with no heterogeneity.

In contrast, Wang *et al.* (2009) reported a significant reduction in methane production as a result of using *Yucca schidigera* extract.

For CH_4/DMI, Yuan *et al.* (2007) and Mao *et al.* (2010) reported that tea saponin lowers CH_4/DMI while other studies reported no significant change in CH_4/DMI compared to the control group as a result of using saponin sources (Santoso *et al.* 2004; Pen *et al.* 2007; Liu *et al.* 2019), although numerical reductions were observed, which likewise to CH_4 production, explains the current result with no heterogeneity. It has been suggested that saponins reduce CH_4 production by reducing protozoa and methanogenic archaea in the rumen (Patra and Saxena, 2009). In the same vein, Jayanegara *et al.* (2014) reported in their meta-analysis that the number of protozoa significantly dropped at higher levels of saponin. Since dihydrogen is the key element in ruminal methanogenesis, a reduced number of protozoa, as a producer of dihydrogen, can lead to lower CH_4 production (Morgavi *et al.* 2010). Protozoa's sensitivity to saponins may be attributed to saponins binding with their membrane sterols (Wina *et al.* 2005).

As noted earlier, given insufficient and lack of uniform data, our meta-analysis did not include an assessment of the number of protozoa. However, Mao *et al.* (2010) reported a significant drop in protozoa number as a result of supplementation with tea saponin while other studies did not observe a significant change in this number (Klita *et al.* 1996; Śliwiński *et al.* 2002; Pen *et al.* 2007; Liu *et al.*2019).

The meta-analysis reported no saponin supplementation effect on ruminal pH, driven by the vast majority of papers in the analysis (Klita *et al.* 1996; Śliwiński *et al.* 2002; Hess *et al.* 2004; Santoso *et al.* 2004; Pen *et al.* 2007; Wang *et al.* 2009; Liu *et al.*2019). However, Yuan *et al.* (2007) and Mao *et al.* (2010) reported lower ruminal pH as a result of saponin supplementation which was driven by an increased ruminal VFA concentration (Mao *et al.* 2010). Our meta-analysis indicated that adding saponin sources did not significantly change acetate concentration, in line with most studies except for Yuan *et al.* (2007). Where as a significant increase was found in effect size for propionate. This increase in propionate concentration was reported by Liu *et al.* (2019) while other studies found a numerical increase in propionate concentration with no significant difference from the control group. These discrepancies appear to be related to the chemical structure and dosage of saponins, diet composition, microbial community, and adaptation of microbiota to saponins (Patra and Saxena, 2009). An expected drop in the acetate-to-propionate ratio was observed while the effect size for this ratio was at a medium level and tended to decrease. Wina *et al.* (2005) suggested that the main effect of saponin on VFA was an increase in propionate proportion and a drop in acetate-to-propionate ratio. They also suggested that this increase in propionate proportion is attributable to lower concentrations of acetate and butyrate since they are among the main products of protozoa fermentation, which is suppressed by saponins (Wina *et al.* 2005). Our meta-analysis showed no significant change in effect size for butyrate although its value was positive. Furthermore, changes in concentrations of acetate and propionate can effectively mitigate CH_4 production since whilst acetate formation is a hydrogen source in the rumen propionate formation is a hydrogen sink, reducing dihydrogen substrate for methanogenesis (Moss *et al.* 2000).

It should be noted that the type of experimental design can influence experimental results. Most studies have used a cross over design which seems to be unsuited for studying the impacts of saponin on CH_4 production and rumen fermentation parameters, particularly because saponins may influence the balance of the rumen microbial community.

CONCLUSION

The findings of the present meta-analysis indicate that CH_4 production tends to decrease and CH_4/DMI is significantly reduced through supplementation of saponin-rich sources in sheep. Besides, supplementation with saponin-rich sources leads to a significant increase in propionate concentration while the acetate-to-propionate ratio tends to decrease, with no significant change observed in ruminal pH and the total concentration of VFA, acetate, and butyrate.

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